

## PHENOLIC COMPOUNDS ON THE POD-SURFACE OF PIGEONPEA, *Cajanus cajan*, MEDIATE FEEDING BEHAVIOR OF *Helicoverpa armigera* LARVAE

PAUL W. C. GREEN,<sup>1</sup> PHILIP C. STEVENSON,<sup>1,2,\*</sup>  
MONIQUE S. J. SIMMONDS,<sup>1</sup> and HARI C. SHARMA<sup>3</sup>

<sup>1</sup>Royal Botanic Gardens  
Kew, Richmond, Surrey TW9 3AB, United Kingdom

<sup>2</sup>Natural Resources Institute  
University of Greenwich  
Chatham Maritime, Kent ME4 4TB, United Kingdom

<sup>3</sup>International Crop Research Institute for the Semi-Arid Tropics  
Patancheru, Andhra Pradesh 502 324, India

(Received May 4, 2002; accepted December 9, 2002)

**Abstract**—A methanol extract of the pod surfaces of *Cajanus cajan*, a feeding stimulant for fifth-instar *Helicoverpa armigera*, was shown to contain four main phenolic compounds. Three of these were identified as isoquercitrin, quercetin, and quercetin-3-methyl ether, by comparing UV spectra and HPLC retention times with authentic standards. The fourth compound was isolated by semipreparative HPLC and determined to be 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid (stilbene) by NMR spectroscopy and mass spectrometry. Quercetin, isoquercitrin, and quercetin-3-methyl did not affect the selection-behavior of fifth-instar *H. armigera*. However, larvae were deterred from feeding on glass-fiber disks impregnated with the stilbene. Furthermore, larvae exposed to quercetin-3-methyl ether consumed significant amounts of both disks. In a binary-choice bioassay, a combination of quercetin-3-methyl ether and the stilbene on one disk and pure quercetin-3-methyl ether on the other disk resulted in increased consumption of both glass-fiber disks by larvae. In contrast, consumption was reduced if the combination was presented to larvae on one disk with purified stilbene on the other disk. *Cajanus cajan* cultivars that varied in their susceptibility to *H. armigera* were surveyed for the presence of the four phenolic compounds. An absence of quercetin and higher concentrations of isoquercitrin than the cultivated variety characterized pod surface extracts of pod-borer-resistant cultivars. In addition, the ratio of the stilbene to quercetin-3-methyl ether was greater in the pod-borer-resistant cultivars. These findings

\* To whom correspondence should be addressed. E-mail. [p.stevenson@kew.org](mailto:p.stevenson@kew.org)

are discussed in relation to the identification of chemical characters that can be used for crop improvement.

**Key Words**—*Cajanus cajan*, podborer, *Helicoverpa armigera*, feeding behavior, stilbene, quercetin-3-methyl ether, quercetin, isoquercitrin.

## INTRODUCTION

*Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a major pest of pigeonpea, *Cajanus cajan* (L.) Millsp. (Fabaceae: Papilionoideae) (Lateef and Reed, 1990; Ranga Rao and Shanower, 1999). Larvae cause substantial damage to plants and reduce the yield of grain by feeding upon flowers and pods (Shanower et al., 1999).

Previous experiments have shown that acetone extracts from the pod surface of a variety of *C. cajan* (ICPL 87) susceptible to pod-borers stimulated the feeding of third-instar *H. armigera* (Shanower et al., 1997). More recently, we showed that hexane, methanol, and water extracts of *C. cajan* (ICPL 87) pods also stimulate feeding of fifth instars, with the methanol extract being most stimulatory (Green et al., 2002). Food selection behavior of *H. armigera* larvae is known to be affected by secondary compounds present in other cultivated species of legumes, such as chickpeas (*Cicer* sp.), in which some compounds act as feeding stimulants, whereas others deter feeding (Simmonds and Stevenson, 2001). Secondary compounds on the surface of pods of *C. cajan* may also modulate the feeding of larvae of *H. armigera*. We now report on the isolation and characterization of some compounds from a methanol extract of the pod surfaces of *C. cajan* (ICPL 87) and describe their effects on the feeding behavior of larvae of *H. armigera*. A comparison of the presence and concentration of these compounds is made among different cultivars of *C. cajan* that vary in their susceptibility to predation by the pod-borer. The implications of these data in understanding the factors that determine why the cultivated genotype of *C. cajan* (ICPL 87) is susceptible to predation by pod-borer larvae are discussed.

## METHODS AND MATERIALS

**Preparation of Extracts.** A methanol extract from a known surface area of freshly collected, 7- to 10-day-old, *C. cajan* pods (ICRISAT accession: ICPL 87) was prepared (at ICRISAT in November 1998) by dipping individual pods into the solvent for 120 sec. The extract was concentrated under reduced pressure and redissolved in methanol so that each 100  $\mu$ l of solvent contained the equivalent extract from 3.46 cm<sup>2</sup> of pod surface, the surface area of a glass-fiber disk. Thus, the concentration of different compounds in an aliquot could be equated to an

area of pod surface to ensure that insects were presented with naturally occurring concentrations during feeding bioassays.

*Isolation and Identification of Compounds from Pod Surface of Pigeonpea (ICPL 87).* Extracts were analyzed and compounds isolated by HPLC (Waters 600E pump; 717 autosampler and 996 photodiode array detector). Aliquots (20  $\mu$ l) were injected onto a reverse-phase column (Merck, Lichrospher 100; 250 mm long, 4 mm ID, RP-18; 5- $\mu$ m particle size, temperature 30°C) and eluted at 1 ml/min using the gradient 25% A: 75% B at  $t = 0$  min to 100% A at  $t = 20$ –30 min, where A is methanol in water and B is 2% acetic acid. Three compounds present in the extract of the pod surfaces were identified by comparison with HPLC profiles of authentic standards (>95% purity, Apin Ltd., Oxford, UK). A fourth, unknown, compound was isolated by manually collecting it as it eluted from a semipreparative reverse-phase column (Merck, Lichrospher 100, 250 mm long, 10 mm ID, RP-18; 10- $\mu$ m particle size, temperature 30°C), with the flow rate set to 4.7 ml/min. The eluent was then dried under reduced pressure.

*NMR Spectroscopy and Mass Spectrometry.* Both 1D and 2D  $^1\text{H}$  NMR spectra of **4** were acquired in  $\text{CDCl}_3$  at 30°C on a Bruker 400 MHz instrument.  $^{13}\text{C}$  NMR data were obtained from the indirectly detected dimension in HSQC and HMBC experiments. Spectra were referenced to residual solvent resonances at  $\delta_{\text{H/C}}$  7.25/77.0 ( $\text{CDCl}_3$ ) relative to TMS. Negative ion first-order MS were recorded using LC-MS (Thermo-Finnigan LCQ) with an electrospray ionization (ESI) source.

*Quantification.* Known concentrations (1, 10, 100, 250, and 500 ppm) of each of the phenolic compounds were injected onto an analytical column and eluted according to the method described above. Peak areas were integrated and compared with the authentic standards (Apin Ltd.), the isolated compound, and an extract of the pod surfaces of ICPL 87 at a known concentration (i.e., 100  $\mu$ l contained the extract from 3.46  $\text{cm}^2$  of pod surface). These data were used to calculate the concentration of compounds in the pod surface extracts.

*Larval Behavior.* Authentic standards (>95% purity; Apin Ltd.) or the isolated compound (>95% purity) were used in the behavioral bioassays. Glass-fiber disks (Whatman GF/A grade, 2.1 cm diam., 3.46  $\text{cm}^2$ ) were impregnated with 100- $\mu$ l aliquots of either a methanol extract of the pod surfaces of ICPL 87 ( $N = 15$ ) or a naturally occurring concentration of one of the four phenolic compounds present in the methanol extract ( $N = 10$ , for each compound). Individual larvae from a control group ( $N = 10$ ) were each presented with two blank disks (no extract or compound). Once dry, disks were weighed and placed into individual 9-cm-diam. plastic Petri dishes with a weighed, untreated disk. Disks were moistened with 100  $\mu$ l of distilled water, as previous experiments had shown that larvae were less likely to feed on a dry disk (Green et al., 2002). Subsequently, one fifth-stadium *H. armigera*, selected from a colony reared at the Royal Botanic Gardens, Kew, UK, according to the methods of Armes et al. (1992) was added to each dish.

Each insect was 24–36 hr into the fifth stadium and had been without food for 2 hr (Simmonds et al., 1990).

Dishes were placed into a growth cabinet 12 hr light at 27°C, 12 hr dark at 20°C, 70% relative humidity. After 24 hr, insects were removed from the dishes and disks were left to dry for a further 24 hr prior to being reweighed to determine the amounts of control and treated disk eaten. The mean amounts of both the control and treated disk eaten by larvae were calculated for all the insects. Feeding indices (FI) were calculated from the amounts of control (C) and treated disks (T) eaten:  $FI = [(C - T)/(C + T)] \times 100$  (Simmonds et al., 1990). Since feeding indices are calculated for each replicate within a treatment, they can be distorted by inclusion of replicates in which larvae consume small amounts of disk material. Therefore, only replicates in which larvae had consumed at least 1 mg of one of the disks were used for the purposes of calculating a FI. This also ensured the inclusion only of those insects taking large bites from a disk, as opposed to those that had merely rasped the surface of the disks without consuming disk material.

*Data Analyses.* The amounts of glass-fiber disk eaten by larvae exposed to different treatments and the feeding indices associated with each treatment were compared with the Mann-Whitney test (MW) (Minitab, v. 12). The quantities of the control and treated disk eaten within a treatment were compared with the Wilcoxon signed rank test (WSR) (Sokal and Rohlf, 1987).

*Comparison of Concentration of Compounds in Other C. cajan Cultivars.* Methanol extracts of a known area of pod surface were prepared from different varieties of *C. cajan* displaying resistance (ICPL 332) or moderate resistance (ICPL 7203-1, ICPL 84060, and ICPL 187-1) to damage caused by podborer larvae. The level of resistance was measured as the proportion of undamaged pods recorded on plants grown in experimental field plots at ICRISAT. These plants were exposed to naturally occurring insect populations (Sharma et al., 2001). The extracts were prepared as described for ICPL 87 and analyzed by HPLC. The concentrations of the four phenolic compounds in the extracts were calculated as described above.

## RESULTS

*Identification of Compounds from Pod Surface of Pigeonpea (ICPL 87).* Methanol extracts of the pod surfaces of ICPL 87 contained isoquercitrin (**1**) ( $R_t = 13.8$  min), quercetin (**2**) ( $R_t = 17.1$  min), and quercetin-3-methyl ether (**3**) ( $R_t = 18.1$  min), respectively (Figure 1).

The UV spectrum of compound **4** ( $R_t = 23.2$  min) recorded in acidic MeOH was typical of a stilbene ( $\lambda_{\max} = 260, 304, 325\text{sh nm}$ ) (Gorham, 1989). Its  $^1\text{H}$  NMR spectrum contained resonances for a double bond in the *E* configuration ( $\delta$  7.82 and 6.82, both 1H, d,  $J = 15.9$  Hz), a monosubstituted phenyl ring ( $\delta$  7.52,

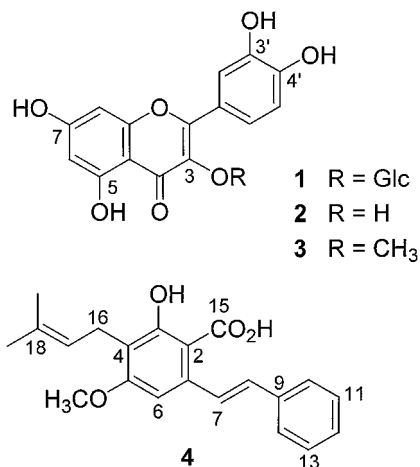


FIG. 1. Structure of phenolic compounds present in a methanol extract of the pod surfaces of *Cajanus cajan*: isoquercitrin (**1**), quercetin (**2**), quercetin-3-methyl ether (**3**) and 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid (**4**).

2H, d,  $J = 7.2$  Hz; 7.37, 2H, t,  $J = 7.5$  Hz, 7.28, 1H, m), and a single aromatic proton at  $\delta$  6.65 (s) indicating the presence of a pentasubstituted phenyl ring. Long-range  $^3J(^1\text{H}-^{13}\text{C})$  correlations observed in HMBC data between the double-bond protons and carbon atoms of both phenyl rings (Table 1) confirmed that **4** was a stilbene. The substituents of the pentasubstituted phenyl ring were identified as carboxyl ( $\delta_{\text{C}}$  174.0), hydroxyl ( $\delta_{\text{H}}$  11.52, br s), prenyl ( $\delta$  3.38, 2H, d,  $J = 7.1$  Hz; 5.21, 1H, m; 1.79, 3H, d,  $J = 0.9$  Hz; 1.68, 3H, d,  $J = 0.9$  Hz), and methoxyl ( $\delta_{\text{H}}$  3.94, s). Their relative locations were determined from long-range  $^1\text{H}-^{13}\text{C}$  correlations in HMBC data (Table 1) and dipolar  $^1\text{H}-^1\text{H}$  connectivities from 1D ROE experiments. ESI-MS data (negative mode) for **4** showed a deprotonated molecule at  $m/z$  337  $[\text{M}-\text{H}]^-$  and a fragment ion at  $m/z$  293  $[\text{M}-\text{H}-44]^-$ , consistent with the loss of  $\text{CO}_2$ . UV, NMR, and MS data allowed the structure of **4** to be confirmed as 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid ( $\text{C}_{21}\text{H}_{22}\text{O}_4$ ), a phytoalexin reported previously from leaves of *Cajanus cajan* challenged with *Botrytis cinerea* (Cooksey et al., 1982).  $^{13}\text{C}$  NMR resonance assignments for this compound were not given in the earlier report and are summarized in Table 1 together with a complete set of  $^1\text{H}$  NMR resonance assignments acquired in  $\text{CDCl}_3$  (previous data given only in acetone- $d_6$  and  $\text{C}_6\text{D}_6$ ).

**Compound Concentrations in Extracts.** Of the four main compounds in the methanol extract of the pod surfaces, **3** occurred at the highest concentration (880 ppm;  $2.46 \mu\text{g}/\text{cm}^2$  of pod surface), followed by **4** (560 ppm;  $1.57 \mu\text{g}/\text{cm}^2$ ), **1** (60 ppm;  $0.17 \mu\text{g}/\text{cm}^2$ ), and **2** (50 ppm;  $0.14 \mu\text{g}/\text{cm}^2$ ).

TABLE 1.  $^1\text{H}$  AND  $^{13}\text{C}$  NMR RESONANCE ASSIGNMENTS ( $\delta$ ) AND COUPLING CONSTANT DATA FOR COMPOUND **4** IN  $\text{CDCl}_3$  AT  $30^\circ\text{C}$ 

Carbon	$^{13}\text{C}$	$^1\text{H}$	HMBC correlations
1	141.7		
2	102.9		
3	162.4		
4	116.8		
5	162.3		
6	103.3	6.65 (s)	C-1 <sup>a</sup> , C-2, C-4, C-5, C-6, C-15 <sup>a</sup>
7	130.4	7.82 (d, $J = 15.9$ Hz)	C-1 <sup>a</sup> , C-6, C-8 <sup>a</sup> , C-9,
8	131.1	6.82 (d, $J = 15.9$ Hz)	C-1, C-7 <sup>a</sup> , C-9 <sup>a</sup> , C-10, 14,
9	137.2		
10, 14	126.8	7.51 (d, $J = 7.2$ Hz)	C-8, C-12,
11, 13	128.8	7.37 (t, $J = 7.5$ Hz)	C-9,
12	127.9	7.28 (m)	C-10, 14
15	174.6		
16	22.2	3.37 (d, $J = 7.1$ Hz)	C-3, C-5, C-17, C-18
17	121.9	5.21 (m)	
18	132.0		
19	17.9	1.79 (d, $J = 0.9$ Hz)	C-17, C-18, C-20
20	26.0	1.68 (d, $J = 0.9$ Hz)	C-17, C-18, C-19
$\text{OCH}_3$	55.8	3.94 (s)	C-5,
3-OH		11.52 (br s)	C-3, C-4, C-2

<sup>a</sup> Weak correlations indicative of two and four bond couplings.

**Larval Behavior.** A methanol extract of ICPL 87 at the naturally occurring concentration per unit area of glass-fiber disk stimulated feeding of fifth-instar *H. armigera* (Table 2). Comparison of the total amount eaten by larvae exposed to either compound **1** or **2** with the total amount consumed in the control shows that neither of these compounds stimulated feeding. In contrast, larvae exposed to **3** and **4** consumed as much of either the compound-treated and/or control disk as occurred when larvae were exposed to the methanol extract (Table 2). However, the effect of **3** and **4** on the food selection behavior of larvae varied. For example, the stilbene showed potent antifeedant activity, whereas **3** did not affect selection behavior.

As the phagostimulatory activity of the methanol extract of ICPL 87 could not be explained by the activity of compounds **1–4** when tested on their own, a combination bioassay was undertaken with the two compounds (**3** and **4**) that resulted in appetitive behavior (Table 3). In this binary-choice bioassay, compound **3** or **4** was applied to one of the disks and exposed to larvae along with a disk treated with both **3** and **4**. The concentration of each compound was the same as occurred in the methanol extract of ICPL 87. Very few larvae exposed to **4** versus the **3** + **4** combination consumed the disks. So the antifeedant activity of **4** masked

TABLE 2. FEEDING BY FIFTH INSTAR *H. armigera*, ON GLASS FIBER DISKS DURING CHOICE BIOASSAYS: EFFECTS OF METHANOL EXTRACT AND COMPOUNDS **1**–**4** ON FEEDING

Extract or compound on treated disk (ppm)	Amount eaten, (mg, mean $\pm$ SEM)			Feeding Index (mean $\pm$ SEM) <sup>b</sup>	N <sup>c</sup>
	Control disk	Treated disk	Control + Treated		
Water control	0.4 $\pm$ 0.11 b	0.5 $\pm$ 0.13 c	0.9 $\pm$ 0.16 b		
ICPL 87 methanol extract	0.3 $\pm$ 0.11 b	4.8 $\pm$ 1.06 * a	5.1 $\pm$ 1.08 a	–88 $\pm$ 4.7 c	10
<b>1</b> (60)	0.3 $\pm$ 0.15 b	0.5 $\pm$ 0.37 c	0.8 $\pm$ 0.48 b		
<b>2</b> (50)	0.6 $\pm$ 0.33 b	0.5 $\pm$ 0.29 c	1.1 $\pm$ 0.61 b		
<b>3</b> (880)	2.7 $\pm$ 1.05 a	2.2 $\pm$ 0.66 b	4.9 $\pm$ 1.35 a	6 $\pm$ 18.4 b	7
<b>4</b> (560)	3.2 $\pm$ 0.86*a	0.9 $\pm$ 0.38 c	4.1 $\pm$ 1.03 a	62 $\pm$ 16.7 a	7

<sup>a</sup> **1** to **4** correspond to the compounds shown in Figure 1.

<sup>b</sup> Where no feeding-index is shown, <5 larvae consumed, on average, >1 mg of both disks.

<sup>c</sup> N = the number of replicates used to calculate the feeding index.

The amounts of control and treated disk eaten, within a treatment, were compared with the Wilcoxon signed rank test. \*  $P < 0.05$ . The amounts of disk eaten and feeding indices were compared between treatments using the Mann-Whitney test, different letters in a column indicate significant differences.  $P < 0.05$ .

the appetitive activity of **3** (Table 3). Larvae exposed to disks treated with **3** and **3** + **4** consumed both disks: thus, in this combination the ability of the stilbene (**4**) to modulate the selection behavior of the larvae has been lost. However, the combinations tested do not enable us to explain the phagostimulant activity of the methanol extract of ICPL 87.

*Comparison of Concentration of Compounds in Other C. cajan Cultivars.* The pod surface extracts of the resistant *C. cajan* varieties were characterized by

TABLE 3. EFFECTS OF COMBINATIONS OF COMPOUNDS **3** AND **4** ON FEEDING BY *H. armigera* LARVAE<sup>a</sup>

Compounds on disks		Amount eaten, (mg, mean $\pm$ SEM)			Feeding Index, (mean $\pm$ SEM) <sup>b</sup>	N <sup>c</sup>
Treatment 1	Treatment 2	Treatment 1	Treatment 2	Treatment 1 + Treatment 2		
<b>3</b>	<b>3</b> and <b>4</b>	2.5 $\pm$ 0.42 a	3.1 $\pm$ 0.70 a	5.6 $\pm$ 0.58 a	1 $\pm$ 16.2	10
<b>4</b>	<b>3</b> and <b>4</b>	0.6 $\pm$ 0.32 b	0.9 $\pm$ 0.28 b	1.4 $\pm$ 0.57 b		

<sup>a</sup> **3** and **4** correspond to the compounds shown in Figure 1. Compounds were applied to disks at their naturally occurring concentrations.

<sup>b</sup> Where no feeding-index is shown, <5 larvae consumed, on average, >1 mg of both disks.

<sup>c</sup> N = the number of replicates used to calculate the feeding index.

The amounts of disk eaten were compared between treatments using the Mann-Whitney test, different letters in a column indicate significant differences.  $P < 0.05$ . There were no differences between the amounts of control and treated disk eaten within each of the two treatments (Wilcoxon Signed Rank,  $P > 0.05$ ).

TABLE 4. CONCENTRATION OF COMPOUNDS PRESENT IN POD SURFACE EXTRACTS OF *C. cajan* CULTIVARS WITH DIFFERENT SUSCEPTIBILITY TO POD-BORER PREDATION

Compound <sup>a</sup>	Degree of resistance to podborer feeding <sup>b</sup> and concentration (ppm), <sup>c</sup> of compounds in different accessions of <i>Cajanus cajan</i>				
	Susceptible	Moderate resistance			Resistant
	ICPL 87	ICPL 7203-1	ICPL 84060	ICPL 187-1	ICPL 332
<b>1</b>	60	253	379	206	412
<b>2</b>	50	ND	ND	ND	ND
<b>3</b>	880	2284	893	774	1092
<b>4</b>	560	1852	738	745	923

<sup>a</sup> **1**, **2**, **3** and **4**, correspond to the compounds shown in Figure 1.

<sup>b</sup> Data from Sharma et al. (2001).

<sup>c</sup> ND = not detected.

the absence of compound **2** and by concentrations of compounds **1** and **4** that differed from those in the extracts of the susceptible cultivar, ICPL 87 (Table 4). The ratio of compound **3** to **4** in the pod-surface extract of the susceptible cultivar (ICPL 87) was 1.6, whereas the ratio in extracts from the less susceptible genotypes varied from 1.2 (ICPL 7203-1, ICPL 332, ICPL 84060) to 1 (ICPL 187-1). A decrease in the proportion of the feeding stimulant **3**, compared to the antifeedant stilbene **4**, could, therefore, contribute to the resistance of the *C. cajan* genotypes to pod-borer larvae (Table 4).

## DISCUSSION

Of the phenolic compounds identified in the methanol extract of the pod surfaces of ICPL 87, quercetin (**2**) is the most widespread in the plant kingdom. This compound is found in many higher plants and frequently occurs in glycosylated forms, such as isoquercitrin (**1**) and rutin (Harborne et al., 1999). Quercetin-3-methyl ether (**3**) has a more restricted distribution and occurs mainly in the leaves, leaf resin, and flowers of the Compositae, Cistaceae, Cyperaceae, and Cactaceae (Harborne et al., 1999). The stilbene (**4**) has previously been reported only from the leaf surfaces of *Cajanus cajan* that had been challenged with the fungus, *Botrytis cinerea* (Cooksey et al., 1982). However, we found that the pod surfaces produced this compound in the apparent absence of fungal infection.

The data presented above show that larvae of *H. armigera* are able to perceive the methanol extract of the pod surfaces, as they consumed more of the disks impregnated with the methanol extract than untreated glass-fiber disks. While a methanol extract of the pod surfaces stimulated feeding, the response of larvae to the phenolics in the extract varied. For example, exposure of larvae to the stilbene



(4) resulted in larvae feeding on the untreated disk, while the other compounds did not affect selection behavior. The fact that larvae did not feed on disks treated with isoquercitrin (1) and quercetin (2) may have been because they were at too low a concentration to elicit a feeding response from larvae of *H. armigera*. Isoquercitrin (1) has been reported to stimulate feeding of *Bombyx mori* (L.) (Hamamura et al., 1962), although it is common for quercetin and derivatives of quercetin not to affect the feeding behavior of Lepidoptera larvae (Lindroth and Peterson, 1988; Faini et al., 1997).

Protection of grain legumes from larvae of Noctuidae, by modulating the levels of phenolic compounds, has been proposed for developing genotypes of chickpea that produce concentrations of isoflavonoids that are deterrent and growth inhibitory to *H. armigera* (Simmonds and Stevenson, 2001). Similarly, development of cultivars of groundnut that produce concentrations of quercetin glycosides and phenylpropanoids deterrent to larvae of *Spodoptera litura* (Fabricius) (Stevenson, 1993) have been suggested. Other phenolic compounds, such as schaftoside (an apigenin-*C*-glycoside) deter feeding by insects, such as the brown planthopper [*Nilaparvata lugens* (Stal)] (Grayer et al., 1994) and affect the growth of planthopper nymphs (Stevenson et al., 1996). Rutin (quercetin-3-*O*-rhamnosyl [1→6] glucoside) similarly deters feeding by *Heliothis zea* (Boddie) and *Helicoverpa armigera* at concentrations in excess of  $10^{-3}$  M (Blaney and Simmonds, 1983).

Single compounds, in isolation, are unlikely to explain completely the interaction between larvae of *H. armigera* and pigeonpea. The response of larvae to combinations of compounds was complex. In a binary choice test, the response of larvae to one disk impregnated with both compound 3 and 4 was determined by the compound, either 3 or 4, present on the other disk: stilbene (4) inhibited feeding on both disks, whereas quercetin-3-methyl ether (3) stimulated feeding. This result shows that quercetin-3-methyl ether (3) could modulate the antifeedant activity of the stilbene (4) at concentrations found in the susceptible cultivar, ICPL 87. However, the larval responses of 1–4 do not explain the phagostimulatory activity of the methanol extract of ICPL 87. It is clear from our results that plant host recognition of pigeonpea by larvae of *H. armigera* is not attributable to any of the individual phenolic compounds isolated in this study. Synergism between two or more compounds can determine host selection by gravid female Lepidoptera (Feeny et al., 1988; Roessingh et al., 1991; Carter et al., 1998). Thus, the interactions between phenolic and/or other compounds could similarly affect the feeding of larvae of *H. armigera* on pigeonpea.

The levels of identified compounds were found to vary among the *C. cajan* cultivars that vary in their susceptibility to pod-borers. Quercetin (2) was absent from all but the susceptible cultivar (ICPL 87). The ratios of quercetin-3-methyl ether (3) to the stilbene (4) were consistent among the three *C. cajan* cultivars (ICPL 332, ICPL 7203-1, and ICPL 84060) that are the least susceptible varieties to pod damage by larvae of *H. armigera* (Sharma et al., 2001) and were less

than occurred in the susceptible variety (ICPL 87). Therefore, an increase in the amount of quercetin-3-methyl-ether, relative to the stilbene, could inhibit feeding inhibition due to the stilbene once the ratio exceeds 1.2.

In conclusion, although a crude methanol extract of the pod surfaces of *C. cajan* stimulates feeding, it also contains phenolics that deter feeding (stilbene, **4**), stimulate consumption (quercetin-3-methyl ether, **3**), or that have no effect on selection or consumption (isoquercitrin, **1** and quercetin, **2**). Further studies are necessary to investigate the effects of different ratios and combinations of these phenolic compounds with the other (nonphenolic) compounds in pod surface extracts on food selection by larvae. Overall, data showed that the absolute amounts of stilbene (**4**) and its concentration relative to other phenolic compounds, especially quercetin-3-methyl ether (**3**), in pigeonpea genotypes could influence predation by larvae of *H. armigera*. Thus, there is a potential for using cultivars that produce high proportions of stilbene, relative to the other phenolics, in selective breeding programs for developing pigeonpea genotypes that are both less susceptible to fungal infection (Cooksey et al., 1982) and more resistant to pod-borers.

*Acknowledgments*—The authors are grateful to Mr. G. Pampapathy, Mr. Rakesh Kumar, Mr. V. V. Rao, Mr. Madhusan Reddy, and Mr. Raja Rao (ICRISAT) and to Miss Iranthi Methathananda (Kingston University, UK) for technical assistance. We thank Drs. N. C. Veitch, and R. J. Grayer (RBG, Kew) for NMR spectra and advice on analysis of phenolic compounds, respectively. Mrs. M. Yule and Mr. M. Gershater are thanked for their help with culturing *Helicoverpa armigera* at Kew. Two anonymous reviewers are also thanked for their comments on the manuscript. This project was funded by a Department for International Development (DFID) Competitive Research Facility Grant [R 7029 (C)] to ICRISAT/NRI/Kew. This work was carried out under a MAFF licence issued to Kew under the Import and Export (Plant Health, Great Britain) Order and the Plant Pest (Great Britain) Order 1993.

## REFERENCES

- ARMES, N. J., BOND, G. S., and COOTER, R. J. 1992. The laboratory culture and development of *Helicoverpa armigera*. Natural Resources Institute Bulletin No. 57. Natural Resources Institute, Chatham, United Kingdom.
- BLANEY, W. M. and SIMMONDS, M. S. J. 1983. Electrophysiological activity in insects in response to antifeedants. COPR Project no. 9, Final Report. ODA, United Kingdom.
- CARTER, M., SACHDEV-GUPTA, K., and FEENY, P. 1998. Tyramine from the leaves of wild parsnip: a stimulant and a synergist for oviposition by the black swallowtail butterfly. *Physiol. Entomol.* 23:303–312.
- COOKSEY, C. J., DAHIYA, J. S., GARRATT, P. J., and STRANGE, R. N. 1982. Two novel stilbene-2-carboxylic acids from *Cajanus cajan*. *Phytochemistry* 21:2935–2938.
- FAINI, F., LABBE, J., SALGADO, I., and COLL, J. 1997. Chemistry, toxicity and antifeedant activity of the resin of *Flourensia thurifera*. *Biochem. Syst. Ecol.* 25:189–193.
- FEENY, P., SACHDEV, K., ROSENBERRY, L., and CARTER, M. 1988. Luteolin-7-*O*-(6''-*O*-malonyl)- $\beta$ -D-glucoside and *trans*-chlorogenic acid: oviposition stimulants for the black swallowtail butterfly. *Phytochemistry* 27:3439–3448.
- GORHAM, J. 1989. Stilbenes and Phenathrenes, pp. 159–196, in P. M. Dey and J. B. Harborne (eds.). *Methods in Plant Biochemistry, Volume 1: Plant Phenolics*. Academic Press, London, United Kingdom.

- GRAYER, R. J., KIMMINS, F. M., STEVENSON, P. C., HARBORNE, J. B., and WIJAYAGUNESSEKERA, H. N. P. 1994. Phenolics in rice phloem sap as sucking deterrents to the brown planthopper (*Nilaparvata lugens*). *Acta Hort* 381:391–394.
- GREEN, P. W. C., STEVENSON, P. C., SIMMONDS, M. S. J., and SHARMA, H. C. 2002. Can larvae of the pod-borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae), select between wild and cultivated pigeonpea [*Cajanus* sp. (Fabaceae)] ? *Bull. Entomol. Res.* 92:45–51.
- HARBORNE, J. B., BAXTER, H., and MOSS, G. P. 1999. *Phytochemical Dictionary: A Handbook of Bioactive Compounds from Plants*. Taylor and Francis, London, United Kingdom.
- HAMAMURA, Y., MATSUURA, K., NISHIDA, J., HAYASHIYA, K., and NAITO, K. I. 1962. Food selection by silkworm larvae. *Nature* 194:754.
- LATEEF, S. S. and REED, W. 1990. Insect pests of pigeonpea, pp. 193–242, in S. R. Singh (ed.). *Insect Pests of Tropical Food Legumes*. John Wiley & Sons, New York.
- LINDROTH, R. L. and PETERSON, S. S. 1988. Effects of plant phenols on southern armyworm larvae. *Oecologia* 75:185–189.
- RANGA RAO, G. V. and SHANOWER, T. G. 1999. Identification and management of pigeonpea and chickpea insect pests in Asia. ICRISAT Information Bulletin no. 57. International Crop Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India.
- ROESSINGH, P., STADLER, E., SCHONI, E., and FEENY, P. 1991. Tarsal contact chemoreceptors of the black swallowtail butterfly, *Papilio polyxenes*: responses to phytochemicals from host- and non-host plants. *Physiol. Entomol.* 16:485–495.
- SHANOWER, T. G., YOSHIDA, M., and PETER, A. J. 1997. Survival, fecundity and behavior of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on pigeonpea and two wild *Cajanus* species. *J. Econ. Entomol.* 90:837–841.
- SHANOWER, T. G., ROMEIS, J., and MINJA, E. M. 1999. Insect pests of pigeonpea and their management. *Annu. Rev. Entomol.* 44:77–96.
- SHARMA, H. C., GREEN, P. W. C., STEVENSON, P. C., and SIMMONDS, M. S. J. 2001. What makes it tasty for the pest? Identification of *Helicoverpa armigera* (Hübner) feeding stimulants and location of their production on the pod-surface of pigeonpea [*Cajanus cajan* (L.) Millsp.]. Competitive Research Facility Project R7029 C, Final Technical Report. DFID, London, United Kingdom.
- SIMMONDS, M. S. J. and STEVENSON, P. C. 2001. Effects of isoflavonoids from *Cicer* on larvae of *Helicoverpa*. *J. Chem. Ecol.* 27:965–977.
- SIMMONDS, M. S. J., BLANEY, W. M., and FELLOWS, L. E. 1990. Behavioral and electrophysiological study of antifeedant mechanisms associated with polyhydroxyalkaloids. *J. Chem. Ecol.* 16:3167–3196.
- SOKAL, R. R. and ROHLF, F. J. 1987. *Biostatistics*, 2nd ed. W. H. Freeman and Co., New York.
- STEVENSON, P. C. 1993. Biochemical resistance in wild species of groundnut to *Spodoptera litura* (Fabr.). *Bull. OILB/SROP* 16:155–162.
- STEVENSON, P. C., KIMMINS, F. M., GRAYER, R. J., and RAVEENDRANATH, S. 1996. Schaftosides from rice phloem as feeding inhibitors and resistance factors to brown planthoppers (*Nilaparvata lugens*). *Entomol. Exp. Appl.* 80:246–249.